REMARKS

CLAIM REJECTIONS - 35 U.S.C. §112

Claim 2 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants herein have amended Claims 2, as suggested by the Examiner, to correct a grammatical inconsistency therein. No new matter has been added. Support for amendment to Claim 2 can be found on page 16, lines 14-16 of the Specification.

CLAIM REJECTIONS - 35 U.S.C. §103

Claims 1-16 were rejected under 35 U.S.C. §103 (a) as obvious over Tautvydas (U.S. Patent 5,795,730) in view of Baker, et al. (U.S. Patent 6,506,803) and Slieman et al. (Applied and Environmental Microbiology, 2001, Vol. 67, Pages 1274-1279).

The Examiner stated that:

> Tautvydas teaches the general concept of simultaneously contacting a contamination containing biological spores in a spore germination medium and exposing said composition to a sterilizant so that the spore containing contaminated solution is decontaminated because vegetative cells emerging through spore germination are sterilized (i., e., killed) by the sterilizant. In said method, *Bacillus subtilis* spores (i.e., a strip mounted with a pre-measured quantity of *Bacillus subtilis* spores) contained in a vial with a spore germination medium are exposed to ethylene oxide (i., e., sterilizant, See Column 3, Line 27-45) and subsequently spore germination rates are spectrophotometrically determined (See Examples 1-2) against a control. Tautvydas' spore germination (SG) medium comprises peroxygen compounds, calcium chloride and other chloride ions but does not contain an enzyme, a surfactant or dipicolinic acid (Column 15, Lines 20-45 and Column 2, Line 48 where H_2O_2 is present as an sterilizant).
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> Baker et al. teach a method and a composition to inactivate/decontaminate bacterial cells and spores by exposing them to an oil-in-water emulsion comprising

water, a surfactant, an oil, an enzyme and a buffer (Abstract, Lines 1-7; Column 5, Lines 12-15; Column 12, Lines 7 -64; Column 18, Lines 18-29; Column 21, Lines 1-32; Column 22, Lines 27-40).

None of the prior art methods cited above teach a composition comprising dipicolinic acid and calcium ions/calcium dipicolanate or calcium chelated with dipicolinic acid to inactivate bacterial spores or decontaminate a solution containing *Bacillus spores*.

Slieman et al. teach that *Bacillus subtilis* spores were germinated when suspended in a freshly prepared solution comprising 60 mM DPA (i.e., dipicolinic acid) and 60 mM $CaCl_2$ at a pH of 8.0 (Page 1275, Column 2, Lines 12-24). In their method to sterilize said contaminants (i.e., *Bacillus subtilis* spores), Slieman et al. further teach that said spores germinated in solution containing DPA-$CaCl_2$ were more sensitive to all wavelengths of UV radiation (Abstract, Lines 13-14). Thus, Slieman et al. already teach the inventive concept of sequential or simultaneous sterilization of bacterial spores by germinating them in a solution containing dipicolinic acid and $CaCl_2$ and a sterilizant, i.e., UV radiation.

One having ordinary skill in the art would have been motivated to modify Tautvydas' teachings according to the teachings from Baker et al. and Slieman et al. to incorporate a surfactant, an enzyme and a solution of dipicolinic acid to Tautvydas' germination medium (SG medium), because all the three prior art methods teach a sterilization/decontamination method to sterilize/decontaminate a solution/material contaminated with bacterial (i.e., *Bacillus* sp.) spores, wherein each one of the prior art reference is essentially substituting one spore gemination method for the other and the three germination methods are functional equivalents to reach other with Slieman et al's method having the advantage of incorporating dipicolinic acid and calcium chloride or calcium dipicolonate in their germination method. While all the three prior art references teach the basic inventive concept to apply a germination solution/medium to simultaneously/sequentially germinate bacterial spores and subsequently kill the vegetative cells resulting from germination of spores, Baker et al. remove the deficiency in Tautvydas' spore germination (SG) medium by teaching addition of an enzyme and a surfactant and Slieman et al. remove the deficiency of combining $CaCl_2$ with dipicolonic acid in methods and compositions from Tautvydas and Baker et al. to germinate the spores and subsequently sterilize the contaminated material with a sterilizant (i.e., UV radiation).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify teachings from Tautvydas and Baker et al. According to the teachings from Slieman et al. to subject bacterial spores to a germination medium comprised of an enzyme, a surfactant, and a mixture of $CaCl_2$ and dipicolonic acid along with subsequent or simultaneous exposure to a sterilizant to decontaminate/sterilize a contaminated material. Thus, Baker et al. remedy the deficiencies in Tautvydas' teachings and Slieman et al. remedy deficiencies in the

> teachings from both Tautvydas and Baker et al.
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> None of the above discussed prior art references teach the exact concentrations or weight ratios for different components (e.g., dipicolinic acid or $CaCl_2$) as claimed in the instant invention. However, adjustment of particular conventional working conditions (e.g., the quantities of each one of the components in a given composition, or method of application of a given composition) is deemed merely a matter of judicious selection and routine optimization of a result-effective parameter, which is well within the purview of a skilled artisan.
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> From the teachings of the references cited *supra*, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Applicants respectfully disagree.

Applicants believe that the Examiner is in error in the Examiner's characterization of the Tautvydas '730 patent. Unlike the present invention that is directed to the use of a germinant to cause germination that renders spores more susceptible to decontamination, Tautvydas '730 discloses a "biological indicator which will evaluate or indicate the effectiveness of a sterilization process in a very short period of time" (col. 2, lns. 21-24). Tautvydas '730 does not use the germinant to aid in the sterilization of the spores, but rather improves on the process where, "microbial spores are exposed to a selected sterilant or sterilizing process and then the survival of any exposed spores is determined by placing the exposed spores in an environment capable of sustaining germination of spores..." (col. 1, lns. 13-24; underlining added). As such, Tautvydas '730 discloses the "steps of i) contacting an indicator comprising microbial spores with a sterilant to give exposed spores, ii) contacting the exposed spores with a medium selected to germinate the spores, iii) determining a rate of germination of the exposed sores in order to assess or determine the effectiveness of sterilization process" (col. 2, lns. 34-40). As Tautvydas '730 uses the "medium selected to germinate the spores"

to "determine the effectiveness of the sterilization process", the Examiner's characterization of Tautvydas '730, that Tautvydas '730 "teaches the general concept of simultaneously contacting a contamination containing biological spores in a spore germination medium and exposing said composition to a sterilizant so that the spore containing contaminated solution is decontaminated because vegetative cells emerging through spore germination are sterilized (i.e., killed) by the sterilizant", is not supported by the disclosure of Tautvydas '730. For example, Tautvydas '730 discloses a "preferred container" that "allows contained spores to come into contact with a sterilant as well as a suitable germinating media" (col. 6, lns. 33-35) which is exemplified in Example 2 as "[*f*]*ollowing* ETO exposure, the growth medium in the ampoules inside the 1264 BI devices was released by crushing the vials (col. 9, lns. 3-5; italics added). As such Tautvydas '730 discloses a method for determining the extent of decontamination using the growth medium, not using the growth medium to decontaminate.

Accordingly, the Examiner's characterization of Tautvydas '730 as teaching "a sterilization/ decontamination method to sterilize/ decontaminate a solution/ material contaminated with bacterial (i.e., *Bacillus* sp.) spores" appears to be inapplicable to the Tautvydas '730 reference. There is no motivation to combine Baker '803 with Tautvydas '730 as stated by the Examiner. Unlike the biological indicator disclosed in Tautvydas '730, Baker '803 discloses a method of preventing and treating microbial infections (title) that uses "an oil-in-water nanoemulsion comprising an oil, an organic solvent, and a surfactant dispersed in an aqueous phase" (col. 5, lns. 28-30). It appears that the Examiner has used hindsight to combine the references.

Additionally, the Slieman et al. reference, characterized by the Examiner to teach "a

sterilization/ decontamination method to sterilize/ decontaminate a solution/ material contaminated with bacterial (i.e., *Bacillus* sp.) spores" (Office Action at page 5, lines 5-7), appears erroneous and to be applied in hindsight. These categories used by the Examiner are not applicable to the Slieman et al. reference. The Slieman et al. reference does not addresses any of these teaching ("a sterilization/ decontamination method to sterilize/ decontaminate a solution/ material contaminated with bacterial (i.e., *Bacillus* sp.) spores") advanced by the Examiner. Slieman et al. discusses "the role of DPA in spore resistance properties" (pg. 1277, col. 2, lns. 11-13) and that "has it become possible to systematically investigate the role of DPA in spore dormancy, resistance, and germination" (pg. 1277, col. 2, lns. 11-13). In light of the fact that "DPA constitutes approximately 10% of *Bacillus subtilis* spore dry weight" (Slieman et al., Abstract, first line), *i.e.*, the DPA is a component part of the spores and not added to the spores, the spores are being analyzed in their natural form. Additionally, Applicants disagree with the Examiner's conclusion that sterilization and decontamination are equivalent. In addition to the distinction of the chemical decontaminant of the present invention, Applicants further disagree with the conclusion that UV radiation is disclosed as a sterilant with the Slieman et al. article as all data indicates some growth.

The Examiner stated that "Slieman et al. teach that *Bacillus subtilis* spores were germinated when suspended in a freshly prepared solution comprising 60 mM DPA (i.e., dipicolinic acid) and 60 mM $CaCl_2$ at a pH of 8.0 (Page 1275, Column 2, Lines 12-24)" (Office Action at pg. 4, 5th paragraph). However, the Slieman et al. disclosure reveals that "[o]ne million spores ... were air dried ... then exposed to different treatments of UV radiation .. [a]fter exposure ... resuspended in 1 ml of a freshly prepared sterile solution of 60 mM DPA and 60 mM $CaCl_2$ (pH 8), and germinated

for 1 h ... and the colonies were counted after overnight incubation..." (pg. 1275, col. 2, lns. 3-24), showing the discussed DPA/$CaCl_2$ solution being used after UV radiation. The Examiner's statement that "Slieman et al. further teach that said spores germinated in solution containing DPA-$CaCl_2$ were more sensitive to all wavelengths of UV radiation (Abstract, Lines 13-14)" appears incorrect. The use of the DPA/$CaCl_2$ solution was used to evaluate the survival of the spores, not enhance the decontamination of the spores. The Slieman et al. article does not disclose the application of DPA solution for UV resistance, rather in Slieman et al. it is the DPA already present in the spores themselves that is evaluated.

Slieman et al. does not disclose that germination makes the spores more susceptible to decontamination or that DPA makes the spores more susceptible to chemical decontamination. In the Slieman et al. reference, the article states that strains FB72 and PS832 were more UV resistant than DPA-less spores of FB108 (page 1277, col. 2, lns. 51-53), indicating that DPA makes these spores more resistant to UV, not more susceptible. On page 1278 (col. 1, lns. 23-25) of the article, the DPA is again implicated in providing a photoprotective effect on air dried spores, particularly in response to UV-B wavelengths. Discussion within the text of the Slieman et al. reference directly related to the cited part of the Abstract by the Examiner details that mutants that cannot germinate via the nutrient pathway, but can with Ca-DPA are slightly more susceptible to UV (page 1278, col. 1, lns. 26-46) , but the mechanism of this occurring is not presented. There is no discussion as to whether this is due to germination, biochemical mechanisms that occur during germination or other factors. The same paragraph also discusses that some components of the Ca-DPA germination pathway may be inactivated by UV, i.e., germination by Ca-DPA could be prevented by UV. It is

also noted that on page 1278 (col. 2, last paragraph), the article states "we have shown that DPA is a major UV resistance factor in spores, especially when air-dried spores on surfaces are exposed to environmentally relevant UV-B wavelengths".

As Slieman et al. is an article, teaching beyond these or other statements within Slieman et al. are speculative. Accordingly, Slieman et al. does not address methodologies of "a sterilization", "decontamination method to sterilize", "decontaminate a solution", or "material contaminated with bacterial (i.e., *Bacillus* sp.) spores" as proposed by the Examiner, and there is neither motivation to combine any teaching of Slieman et al. with Tautvydas '730, nor are there any teachings of Slieman et al. that spores germinated in solution containing DPA-$CaCl_2$ were more sensitive to all wavelengths of UV radiation. The "inventive concept of sequential or simultaneous sterilization of bacterial spores by germinating them in a solution containing dipicolinic acid and $CaCl_2$ and a sterilizant, i.e., UV radiation" (Office Action at pg. 4, 5th paragraph) is not disclosed in Slieman et al.

Applicants respectfully request reconsideration of the instant claims, withdrawal of the rejections cited in the Office Action, and allowance of the instant claims.

The Examiner is invited to contact the attorney of record, listed below, with any questions or other matters to advance the present application.

Respectfully submitted,

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